

Kindly insert the amended substitute paragraphs provided in Appendix 1 attached hereto. Marked-up copies of the amended paragraphs are provided in Appendix 2.

Kindly insert the Sequence Listing submitted herewith.

**REMARKS**

The amendments submitted above are to insert reference to SEQ ID Nos, and do not add any new matter.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Submitted herewith is a request for a 1-month extension of time, to allow timely filing up to and including May 28, 2002 (May 27, 2002 falling on a Federal holiday), and a check for the fee for that extension.

No additional fee is believed due in connection with this communication. However, if any additional fee is due, kindly charge the appropriate amount to Deposit Account 50-0872.

Respectfully submitted,

Date 28 May 2002

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**Appendix 2: Marked-up copies of amended paragraphs**

Paragraph at p.28, lines 8-12

FIGURE 1 is a flow schematic showing the manipulations necessary to convert pT0021, an arsenite inducible vector containing the luciferase gene, into pTHA or pTM, two ars inducible vectors, or pTMLac, a lactose-inducible promoter. a) Vector pTHA contains BamH I and Sal I cloning sites and a downstream HA epitope tag. (SEQ ID NOs: 148 and 149) b) (SEQ ID NOs: 150, 160, 161, 162, and 163) and c) Vector pTM and pTMLac contain Bam HI and Hind III cloning sites and no HA epitope tag. (SEQ ID NOs: 154, 164, and 165)

Paragraphs at p.30, lines 19-24

FIGURE 9 shows the identification of PT48 as *S. aureus* DNA-directed DNA polymerase III beta subunit protein from the Genbank database (accession number: 1084189). (fragments of SEQ ID NO: 167)

FIGURE 10 shows the nucleotide (SEQ ID NO: 166) and amino acid (SEQ ID NO: 167) sequences of *S. aureus* DnaN.

Paragraph at p.60, line 21 to p.63, line 3

5'-gatcccggtcgaccaagcttTACCCATACGACGTCCCAGACTACGCCAGCTGA-3' (SEQ ID NO: 148) (where upper case letters denote the nucleotide sequence of the HA tag); the antisense strand HA tag sequence (with a HindIII cloning site) is:

5'-agctTCAGCTGGCGTAGTCTGGGACGTCGTATGGGTAAagcttggtcgaccgg-3' (SEQ ID NO: 149) (where upper case letters denote the sequence of the HA tag). The two HA tag oligonucleotides were annealed and ligated into pT0021 vector which had been digested with BamHI and HindIII. This manipulation resulted in replacement of the lucFF

gene by the HA tag. This modified shuttle vector containing the arsenite inducible promoter, the *arsR* gene, and HA tag was named pTHA. A diagram outlining our modification of pT0021 to generate pTHA is shown in Fig. 1A.

The shuttle vector pT0021 was also modified as below to suit our specific application. Two oligonucleotides were synthesized. The sense strand sequence (with XhoI cloning site) is: 5'-AATTCTCGAGTAAAATAACAT-3' (SEQ ID NO: 150); the antisense strand sequence (with a BamHI cloning site) is:

5'-CGGGATCCGCCTCCTTTTCTCAACAGTCACCTGATTT -3'. (SEQ ID NO: 151)

The two oligonucleotides were used for polymerase chain reaction (PCR) amplification of pT0021 vector. The PCR product was gel purified using the Qiagen kit as described, and digested with XhoI and BamHI. The digested PCR product was again gel purified, ligated into XhoI and BamHI digested pT0021 vector, and used to transform *E. coli* bacterial strain DH103 (as described above). This manipulation results in the construction of a pT0021-intermediated vector containing a RBS sequence located immediately upstream of the BamHI cloning site. Two other oligonucleotides were synthesized. The sense strand sequence (with BamHI cloning site) is:

5'-CGGGATCCATGAGGGGTTCGAAGACG-3' (SEQ ID NO: 152); the antisense strand sequence (with a HindIII cloning site) is: 5'-CCCAAGCTTACAATTTGGACTTTC -3' (SEQ ID NO: 153). The two oligonucleotides were used for PCR amplification of pT0021-intermediated vector. The PCR product was gel purified and digested with BamHI and HindIII. The digested PCR product was then gel purified as described, ligated into BamHI and HindIII digested pT0021-intermediated vector, and used to transform *E. coli* bacterial strain DH103. This modified shuttle vector containing the ATG of the *lacFF* gene located immediately downstream of the BamHI cloning site was named pTM. A diagram outlining our modification of pT0021 to generate pTM is shown in Fig 1B.

As another example of inducible promotor, the arsenite-inducible promotor and the *asrR* gene from the pTM vector were replaced by a lactose-inducible promotor and the *lacR* gene from *Staphylococcus aureus*. The *S. aureus* gene encoding for the repressor of the *lac* operon (*lacR*) is found immediately upstream of the promoter-proximal end of the *lacA-G* genes. Two oligonucleotides corresponding to a 2.18kb-DNA region

encompassing the lacR and the lac operon promoter region were synthesized. The sense strand sequence is : 5'-ccgctcgagCTCCAAATTCCAAAACAG-3' (SEQ ID NO: 154) (with a XhoI cloning site, ctcgag) ; the antisense strand sequence is: 5'-cgggatccAATAAGACTCCTTTTAC-3' (SEQ ID NO: 155) (with a BamHI cloning site, ggatcc). These two oligonucleotides were used for the PCR amplification of *Staphylococcus aureus* DNA. The PCR product was gel purified and digested with XhoI and BamHI. The digested PCR product was also gel purified, ligated into XhoI and BamHI-digested pTM vector, and used to transform *E. coli* bacterial strain DH10 $\beta$ . In the resulting vector, pTMLac, the firefly luciferase (lucFF) expression is under the control of the *S. aureus* lac operon promoter/operator. Recombinant pTMLac clones were picked and the sequence integrity of the 2.18kb-lac operon region (lacR + lac promoter) was verified directly by DNA sequencing. A diagram outlining the pTMSLac vector characteristics is shown in Fig 1C.

#### **Cloning of ORFs with a Shine-Dalgarno sequence.**

Each ORF, encoded by Bacteriophage 44AHJD, larger than 33 amino acids and having a Shine-Dalgarno sequence upstream of the initiation codon was selected for functional analysis for bacterial inhibition. In total, 31 ORFs were selected and screened as detailed below. A list of these is presented in Fig 4A. As outlined in Fig 2A, each individual ORF, from initiation codon to last codon (excluding the stop codon), was amplified from phage genomic DNA using the polymerase chain reaction (PCR). For PCR amplification of ORFs, each sense strand primer targets the initiation codon and is preceded by a BamHI restriction site (5'cgggatcc3') (portion of SEQ ID NO: 155) and each antisense oligonucleotide targets the pentultimate codon (the one before the stop codon) of the ORF and is preceded by a Sal I restriction site (5'gcgtcgaccg3') (SEQ ID NO: 156). The PCR product of each ORF was gel purified and digested with BamHI and Sall. The digested PCR product was then gel purified using the Qiagen kit as described, ligated into BamHI and Sall digested pTHA vector, and used to transform *E. coli* bacterial strain DH10 $\beta$  (as described above). As a result of this manipulation, the HA tag is set inframe with the ORF and is positioned at the carboxy terminus of each ORF (pTHA/ORF clones).

Recombinant pTHA/ORF clones were picked and their insert sizes were confirmed by PCR analysis using primers flanking the cloning site. The names and sequences of the primers that were used for the PCR amplification were: HAF: 5'TATTATCCAAACTTGAACA3' (SEQ ID NO: 157); HAR: 5'CGGTGGTATATCCAGTGATT3' (SEQ ID NO: 158). The sequence integrity of cloned ORFs was verified directly by DNA sequencing using primers HAF and HAR. In cases where verification of ORF sequence could not be achieved by one pass with the sequencing primers, additional internal primers were selected and used for sequencing.

Paragraph at p.65, lines 11-13

MKIKVKKEMRLDELIKWARENPDL SQGKIFFSTGFSDGFVRHPNTNKCSTSSFIPIDIPFIV  
DIEKEVTEETKVDR LIELFEIQEGDYNSTLYENTSIKECLYGRCVPTKAFYILNDDL  
TMTLIWKDGELLV. (SEQ ID NO: 159)